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DMPP is ineffective at mitigating N₂O emissions from sheep urine patches in a UK grassland under summer conditions



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ABSTRACT

Nitrification inhibitors are a potential technology to mitigate N₂O emissions from the urine patches of grazing animals. At present, there is limited information regarding the efficacy of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in reducing N₂O emissions from ruminant urine patches, as opposed to the well-studied nitrification inhibitor, dicyandiamide. In practical terms, urine patches would be deposited to soil at various times following the application of a nitrification inhibitor to soil. We hypothesised that the effectiveness of DMPP in reducing cumulative N₂O emissions would decrease the longer the time since DMPP application. This study utilised an automated closed chamber technique, to monitor fluxes of N₂O from sheep urine patches (725 kg N ha⁻¹; 150 ml; 300 cm²) deposited to a Eutric Cambisol, where DMPP was applied (1 kg ha⁻¹) on the same day, 2 weeks before and 4 weeks before urine application. Fluxes were monitored continuously from 4 weeks before, to 9 weeks after, urine application. DMPP was found to be ineffective at reducing cumulative N₂O emissions and 9-week urine-N₂O emission factors when applied at the same time as the sheep urine, although a low number of replicates were used in this study. Some effect of DMPP in delaying the accumulation of soil NO₃⁻ was observed, with effects being greater the shorter the time since DMPP application. The temporal dynamics of N₂O fluxes were also altered where DMPP was applied on the same day as the urine. Heterogeneity in soil conditions were deemed responsible for the large spatial variability of N₂O emissions observed in this study. The use of Rhizon samplers were useful for detecting spatial variability within the soil solution directly beneath the urine patches (within the flux chambers), which may not have been detected within duplicate urine patches (outside the chambers), where soil sampling was conducted. Further work is required to determine the loading rate and duration of efficacy of DMPP to reduce emissions from urine patches under temperate summer conditions.

1. Introduction

The sustainable management of livestock excreta will form an important component of reducing agricultural greenhouse gas (GHG) emissions, especially under projected increases in global meat consumption (Davidson, 2009). Urine patches deposited to pastures are known hotspots for N cycling, where microbial transformations (e.g. nitrification and denitrification) can result in elevated N₂O emissions (De Klein and van Logtestijn, 1994; Carter, 2007). Excreta (dung and urine) deposited to pasture soils account for ca. 40% of the global N₂O emissions arising from animal production systems (Oenema et al., 2005).

The use of nitrification inhibitors within grazed pastures offers a potential means of reducing N losses whilst promoting nitrogen use efficiency (Di and Cameron, 2002, 2007; Cardenas et al., 2016). By delaying the first and rate-limiting step of nitrification (the oxidation of

NH₄⁺ to NO₂⁻; Chaves et al., 2006; Fiencke and Bock, 2006; Benckiser et al., 2013), nitrification inhibitors enable N to persist in the soil in the ammoniacal form for longer. This increases opportunity for plant acquisition of applied N, reduces NO₃⁻ leaching and can reduce N₂O emissions from both nitrification and denitrification processes. Two of the most widely researched nitrification inhibitors are dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP; Liu et al., 2013), where the latter is a newer product, developed by BASF (Ludwigshafen, Germany; Barth et al., 2001; Zerulla et al., 2001). Reviews on the efficacy of nitrification inhibitors have revealed a greater effectiveness of these products in reducing N₂O emissions from grasslands, in comparison to other land use types (Akiyama et al., 2010; Abalos et al., 2014; Gilsanz et al., 2016).

There have been a greater number of studies exploring the effect of DCD in reducing N₂O emissions from urine patches compared to DMPP (e.g. Di and Cameron, 2003; De Klein et al., 2011; Misselbrook et al.,

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2014; Bell et al., 2015). DMPP has been suggested for use in pasture soils as it has a lower phytotoxicity and it has slower degradation rates in soil in comparison to DCD (Weiske et al., 2001; Zerulla et al., 2001). DMPP has been shown to be effective in reducing N_2O emissions from fertilisers (Linzmeier et al., 2001; Menéndez et al., 2012; Bell et al., 2015) and livestock slurry (Dittert et al., 2001; Hatch et al., 2005; Merino et al., 2005), but there are few studies investigating DMPP efficacy where ruminant urine is the nitrogen source (e.g. Di and Cameron, 2011, 2012; Gilsanz et al., 2016). Therefore, further information is required to determine the suitability of DMPP as a means of reducing N_2O emissions from grazed grasslands. When applied at the same time as a cattle urine application ($1000 \text{ kg N ha}^{-1}$), a liquid DMPP (5 kg ha^{-1}) application reduced N_2O emissions by 66% (Di and Cameron, 2012). Misselbrook et al. (2014) and Barneze et al. (2015) investigated the N_2O reduction efficacy of pyrazole derivatives (which are structurally similar to DMPP; highest application rate of 3.76 kg ha^{-1}) applied to cattle urine under UK summer conditions, however, no significant reduction in N_2O emissions were observed. The nil effect may have been related to higher temperatures under summer conditions, or an effect of the loading rate of the applied compounds. DMPP performance has been found to be best under cold and wet conditions (Menéndez et al., 2012), therefore, whether DMPP is effective under warmer soil conditions is still unclear.

This study was carried out to determine whether DMPP is effective at reducing N_2O emissions arising from pasture soils influenced by sheep urine, under UK summer conditions. Given that sheep will deposit urine at various times following an application of DMPP, and that cost and labour associated with application will increase with the need for repeated applications, we wanted to assess the duration of DMPP efficacy in reducing N_2O emissions. We used a high frequency automated GHG monitoring system to evaluate the efficacy of a liquid DMPP application in reducing N_2O emissions from sheep urine applied immediately after, 2 weeks after and 4 weeks after DMPP application. We hypothesised that the efficacy of DMPP in reducing N_2O emissions would decrease with the greater the time-since application, due to degradation and immobilisation of the nitrification inhibitor within the soil. The experimental design also allowed i) an assessment of different approaches to measuring N and C dynamics within the urine patch, both inside (Rhizon samplers) and outside (standard soil extractions) of the chambers, and ii) an insight into spatial and temporal variability of soil derived N_2O emissions.

2. Materials and methods

2.1. Field site

The study site was established on a lowland grazed grassland at Henfaes Research station, Abergwyngregyn, North Wales ($53^{\circ}14'N$, $4^{\circ}01'W$). The soil at the site is classified as a Eutric Cambisol, and is of glacial till (deposited ca. 10 000 years ago) in origin. The recent history includes moderate fertiliser applications ($60\text{--}80 \text{ kg N ha}^{-1}$ since 2002, and 120 kg N ha^{-1} from 1990 to 2002), light grazing by Welsh Mountain ewes ($2\text{--}3$ livestock units ha^{-1}) and reseeded in 1990 with a *Lolium perenne* L. and *Trifolium repens* L. mix. To prevent the effects of recent livestock excretal depositions on monitored gas fluxes, livestock were excluded from the study site five months prior to treatment application.

2.2. Soil sampling and analysis

To provide the background characteristics (Table 1) of the study site, soil was sampled (0–10 cm, 1.5 cm diameter) in triplicate, at the block level ($n = 3$). Soil bulk density was measured by inserting a 100 cm^3 metal ring into the ground (0–5 cm), oven-drying the removed soil core (105°C ; 24 h), and sieving ($< 2 \text{ mm}$) the dry soil to remove and weigh stones. Gravimetric moisture content was determined by

Table 1

Properties of the Eutric Cambisol at the field site receiving urine and DMPP applications, at the time of the first DMPP Application. Values represent means \pm SEM ($n = 3$) and results are reported on a dry weight basis.

Eutric Cambisol properties (0–10 cm)	
Texture	Sandy Clay Loam
Bulk density (g cm^{-3})	1.08 ± 0.05
Gravimetric moisture content (%)	21.5 ± 0.9
Organic matter (%)	9.99 ± 0.32
pH	5.91 ± 0.03
EC ($\mu\text{S cm}^{-1}$)	32.7 ± 0.9
Total C (%)	3.99 ± 0.01
Total N (%)	0.34 ± 0.01
C:N ratio	11.6 ± 0.2
Dissolved organic C (mg C kg^{-1})	170 ± 25
Total dissolved N (mg N kg^{-1})	23.1 ± 2.8
Microbial biomass C (g C kg^{-1})	2.44 ± 0.30
Microbial biomass N (mg N kg^{-1})	171 ± 10
Extractable NO_3^- (mg N kg^{-1})	3.97 ± 0.45
Extractable NH_4^+ (mg N kg^{-1})	3.23 ± 0.99
Extractable P (mg P kg^{-1})	3.37 ± 0.44
Exchangeable Na (mg kg^{-1})	43.8 ± 1.1
Exchangeable K (mg kg^{-1})	140 ± 12
Exchangeable Ca (g kg^{-1})	1.07 ± 0.09

oven drying soil (105°C ; 24 h) and organic matter was determined by loss-on-ignition (450°C ; 16 h; Ball, 1964). Soil pH and electrical conductivity (EC) were measured with standard electrodes, submerged in 1:2.5 (w/v) soil-to-distilled water suspensions. The total soil C and N content of oven-dried and ground samples were determined using a TruSpec[®] Analyzer (Leco Corp., St. Joseph, MI); pasture foliar C and N contents, before and during the study, were also analysed using the same instrument. Dissolved (1:5; w/v; soil-to-0.5 M K_2SO_4) total C and N concentrations in soil were determined by the method of Jones and Willett (2006), using a multi N/C 2100S analyser (AnalytikJena, Jena, Germany). The CHCl_3 -fumigation-extraction method of Voroney et al. (2008), was used to determine microbial biomass C and N, where the resulting C and N in the extracts were analysed as described previously, using K_{EC} and K_{EN} correction factors of 0.35 and 0.5, respectively. Concentrations of P, NO_3^- and NH_4^+ were determined in 0.5 M K_2SO_4 soil (0–5 cm) extracts using colorimetric methods described in Murphy and Riley (1962), Miranda et al. (2001) and Mulvaney (1996), respectively. Exchangeable cations (Na, K, Ca) were measured using a Sherwood Model 410 flame photometer (Sherwood Scientific Ltd., Cambridge, UK) in 1 M NH_4Cl extracts (1:5, w/v, soil-to-1 M NH_4Cl).

2.3. Sheep urine collection and analysis

Welsh Mountain ewes ($n = 6$) were housed in individual pens, containing slatted plastic flooring specifically designed for sheep (Rimco Ltd., Yorkshire, UK). The pen flooring was slightly raised above the ground, allowing room for urine collection trays. The sheep were allowed free access to water and provided with a diet (ad libitum) of freshly cut pasture similar in composition to the field site. During the urine collection period individual urine volumes were recorded and the samples were filtered ($0.45 \mu\text{m}$) and frozen prior to use. The urine collected from all sheep was bulked (to provide sufficient volume of homogenous composition) and thoroughly mixed before application to the soil. Total dissolved organic C, total N, P, NO_3^- , NH_4^+ and cations in the sheep urine were analysed as described for the soil extracts, and the urea content was determined via the method of Orsonneau et al. (1992).

2.4. Experimental design, treatments and application dates

The study was laid out in a randomised block design, consisting of five treatments ($n = 3$) as follows: 1) no urine application (control), 2)

sheep urine only, 3) sheep urine plus DMPP applied at the same time, 4) sheep urine plus DMPP applied 2 weeks before urine application, and 5) sheep urine + DMPP applied 4 weeks before urine application, hereafter referred to as NU, SU, SU + DMPP, SU + DMPP 2, and SU + DMPP 4, respectively. For each treatment, urine patches were duplicated next to each flux chamber, allowing one patch for monitoring greenhouse gases from undisturbed soils and another for soil sampling. The pooled sheep urine (150 ml at 14.5 g N l^{-1}) was poured onto respective plots using a fixed template as an area guide (300 cm^2), to represent an average sheep urine volume and patch size (Doak, 1952). This resulted in a urine-N loading rate of 725 kg N ha^{-1} and a total of 2.17 g urine-N applied to the chambers receiving urine applications. Liquid applications of DMPP (250 ml; $0.1 \text{ g DMPP l}^{-1}$) were sprayed (a normal nitrification inhibitor application method for grazed grasslands; Di and Cameron, 2011) by hand onto respective plots (0.25 m^2 , across the chamber basal area), at a frequently used equivalent rate of $1 \text{ kg DMPP ha}^{-1}$ (Zerulla et al., 2001). The first DMPP treatment application to respective plots (4 weeks before urine application; SU + DMPP 4) took place on 29/6/2015, the second DMPP treatment application (2 weeks before urine application; SU + DMPP 2) was applied on 13/7/2015, and the final DMPP treatment application (SU + DMPP) took place on 27/7/2015. Following this, sheep urine was applied to all plots except for the control on the same date (27/7/2015) as the final DMPP application.

2.5. Greenhouse gas emission monitoring

An automated high frequency (8 flux measurements per day/night cycle) greenhouse gas monitoring system (Queensland University of Technology, Institute for Future Environments, Brisbane, Australia), as described in Scheer et al. (2014), was used to measure fluxes of N_2O from the urine-treated soils. As the automated system consisted of 12 chambers only (i.e. enough for 4 treatments, where $n = 3$), fluxes of N_2O from the NU treatment were measured manually using the conventional static chamber technique. Here, 1 flux measurement was made as close to daily as possible, which on the majority of sample dates ($> 86\%$) was taken between 10 am and 12 noon as suggested in Rochette et al. (2015), with the remainder being sampled between 12 noon and 2 pm. Fluxes of N_2O were expected to be lower and less temporally variable from the NU treatment, compared to urine treatments, as no N was applied. We, therefore, assumed the difference in measurement techniques to cause minimal bias in the results.

For the automated chambers, stainless steel chamber bases (0.25 m^2 basal area) were inserted into the soil (10 cm depth), and chambers ($50 \text{ cm} \times 50 \text{ cm} \times 15 \text{ cm}$) were clamped to the bases. The chambers opened and closed via pneumatic actuators, where one block of chambers would close sequentially every 1 h. While one block of chambers were closed, the other two blocks of chambers were open, allowing ambient conditions to be restored within the chambers. Chamber headspace gas samples were pumped (ca. 200 ml min^{-1}) to a sampling unit, through Teflon tubing. The sampling unit housed a LI-COR LI-820 non-dispersive infrared gas analyser (LI-COR, St Joseph, MI, USA) to measure CO_2 . Samples were then passed through an Ascarite (sodium hydroxide coated silica) filter before being pumped to a gas chromatograph (SRI 8610C, Torrance, USA), equipped with a ^{63}Ni electron capture detector (ECD) and flame ionization detector (FID) to measure N_2O and CH_4 concentrations, respectively. Over the 1 h chamber closure period, each chamber ($n = 4$) was sampled at 3 min intervals followed by a calibration standard (500 ppb N_2O ; 880 ppm CO_2 ; 3 ppm CH_4 ; $\pm 2\%$ of the certified value; BOC Gases Ltd., Liverpool, UK). For each chamber, this results in headspace concentration measurements once every 15 min, over the course of 1 h. A full cycle takes 3 h to complete, resulting in (where uninterrupted measurement occurs) 8 flux measurements per 24 h.

The manual gas samples from the NU treatment were taken by placing circular polypropylene chambers (ca. 26 cm in height) onto

collars (26 cm diameter), inserted to a depth of 10 cm. The chambers were fitted with a re-sealable vent to allow pressure equalisation when placing chambers onto bases, and were fitted with Suba-Seals® (Sigma, Gillingham, UK) to allow headspace gas sampling. Headspace samples were taken with a syringe every 15 min over the period of 1 h, to match the automated system. Samples were stored in pre evacuated 20 ml glass vials, before being analysed on a PerkinElmer 580 Gas Chromatograph served by a TurboMatrix 110 auto sampler (PerkinElmer, CT, USA). The system contained two Elite-Q PLOT megabore capillary columns, with split injection. One column was linked to an ECD (375°C), and the other to an FID (350°C) with methaniser, and the oven temperature was 50°C . Fluxes from the automated system were measured for a total of 13 weeks, (beginning 4 weeks before urine application and ending 9 weeks after urine application), and fluxes which were sampled manually were measured for 9 weeks following urine application. Prior to the start of the experiment, standard gases were analysed by both GCs (laboratory and field system) and shown to be not significantly different (data not shown).

2.6. Ancillary measurements

To support the emission measurements, several ancillary measurements were made. Soil cores (0–5 cm) were taken from replicated urine patches on several dates throughout the experiment. Gravimetric moisture content measurement and 1:5 (w/v) soil-to-0.5 M K_2SO_4 extractions were conducted on the resulting soil samples. The extracts were analysed for NO_3^- , NH_4^+ , extractable dissolved organic C and total N, as described previously. In order to allow sampling of the soil solution from within the chambers in a non-destructive manner, Rhizon® suction samplers (2.5 mm diameter, 5 cm porous part, 12 cm length tubing; Rhizosphere Research Products, Wageningen, Netherlands) were inserted into the centre of the chamber plots, at a 45° angle in relation to the soil surface, and were in situ four weeks before urine application. 1 ml soil solution samples were collected periodically from the Rhizons through a needle inserted into an evacuated 9 ml plastic container, when soil conditions were moist enough for successful sample collection. The soil solution samples were also analysed for NO_3^- , NH_4^+ , dissolved organic C and dissolved total N, as described previously. Soil moisture sensors (Acclima SDI-12 digital TDT® sensors; Acclima Inc., ID, USA) were inserted diagonally through the urine patch (but were placed in situ several weeks prior to urine application) within the chambers, in order to measure soil moisture non-destructively. The pasture foliar N content was measured within the chambers at block level prior to urine application, and pasture biomass and foliar N content was measured in each chamber at 3, 6 and 9 weeks following urine application. The grass was also cut in the duplicated plots at the same time as the chambers to ensure consistency. Air temperature was monitored inside and outside the chambers using Thermochron iButtons® (iButtonLink, LLC, WI, USA) logging temperature every 1 h. Rainfall, soil (0–10 cm) and air temperature were monitored hourly at a weather station near to the field site.

2.7. Data processing and statistical analysis

Fluxes of N_2O were calculated from each chamber as described in Scheer et al. (2014). The linear slope of N_2O concentration over time was comprised of either three or four data points. Cumulative N_2O emissions were calculated by trapezoidal integration (from the point of urine application onwards). As urine was applied to only a 0.03 m^2 area of soil, out of a possible 0.25 m^2 enclosed by the chamber, cumulative emissions were corrected in a similar fashion to Marsden et al. (2016). Briefly, the results for all the treatments with a urine application were corrected for the area unaffected by urine within the chamber (0.22 m^2), by deducting the cumulative N_2O emissions arising from

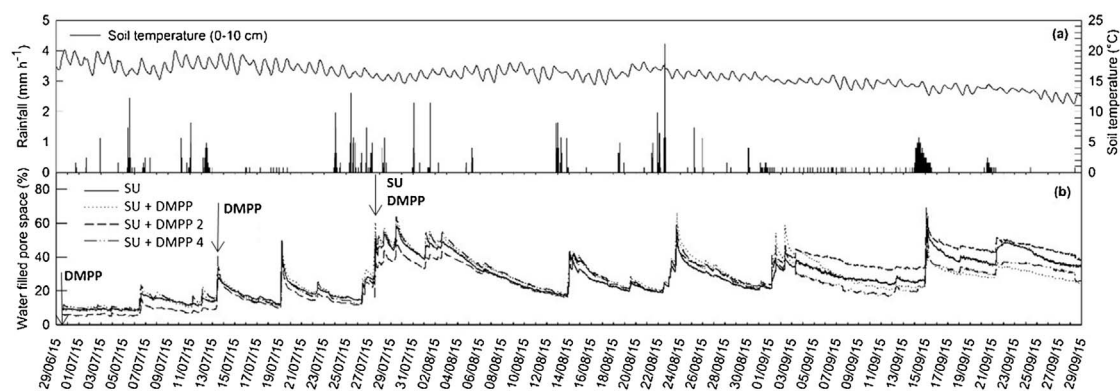


Fig. 1. Rainfall, soil temperature and soil water-filled pore space during the course of the field trial. Hourly rainfall and soil (0–10 cm) temperature are shown in panel a) and water-filled pore space within the automated chambers are displayed in panel b). Arrows indicate the timing of either sheep urine (SU) and DMPP applications, the date of measurements are displayed in dd/mm/yy format and legends apply to each respective panel only.

the NU treatment over 0.22 m^2 . This provided emissions arising directly from the urine patch area (0.03 m^2), where results were then up-scaled to express the direct urine patch cumulative N_2O emissions over 1 m^2 . These corrected values were then used to calculate the emission factors, using the following equation:

$$\text{EF} = (\text{treatment } \text{N}_2\text{O-N} - \text{control } \text{N}_2\text{O-N}) / \text{Total N applied} \times 100\% (1)$$

Differences in cumulative N_2O emissions were analysed by a one way ANOVA, with Tukey's post-hoc test, after assessing the normality (Shapiro Wilk test) and homogeneity of variance (Levene's test) assumptions. The ANOVA was conducted between treatments NU, SU and SU + DMPP only, due to the extreme variability observed in the SU + DMPP 2 and SU + DMPP 4 treatments. For the same reasons, the urine patch 9-week N_2O emission factors were only compared statistically between SU and SU + DMPP, by the use of a *t*-test.

An analysis was conducted to determine the best time within a 24 h period to sample chambers in a manual sampling campaign, in order to best represent emissions calculated from the automated system. Days without a full 8 flux measurements were removed from the dataset and cumulative N_2O emissions were then calculated for the automated sampling dataset alongside eight different sampling-time scenarios (00:00–03:00, 03:00–06:00, 06:00–09:00, 09:00–12:00, 12:00–15:00, 15:00–18:00, 18:00–21:00 and 21:00–24:00), across all chambers ($n = 12$). The cumulative emissions from the simulated sampling campaigns were compared to that arising from the automated chamber dataset, to determine how accurate emission measurements would have been if taken within the sampling time windows tested.

ANOVA followed by Tukey's post-hoc test was conducted for extractable NH_4^+ , NO_3^- , N and dissolved organic C at each sampling point, following log transformation of the data, in order to satisfy the normality and homogeneity of variance assumptions (same tests used as above). The soil solution NH_4^+ , NO_3^- , N and dissolved organic C had a variable n , ranging from 1 to 3, due to successful sample collection being dependent on soil conditions being moist enough. Therefore, numerical trends in the soil solution NH_4^+ , NO_3^- , total N and dissolved organic C data were reported rather than statistical differences. The soil solution mineral N data allowed determination of whether N transformations were occurring at similar rates in the chambers and the duplicated plots used for the soil coring, as the Rhizon samplers were located directly inside the chambers. Pasture biomass and foliar N content were also analysed by ANOVA, as above, on each of the three biomass cutting dates. All statistical analyses were conducted in Minitab 17.0 (Minitab Inc., State College, PA, USA).

3. Results

3.1. Urine characteristics

The composite sheep urine sample contained 14.5 g N l^{-1} and 28.7 g C l^{-1} , which resulted in an equivalent N application rate of 725 kg N ha^{-1} in all applied urine patches. The N content of the composite urine sample was high, although within reported ranges for sheep (Hoogendoorn et al., 2010). The high N content within the urine may have been a consequence of the sheep being fed ad libitum, whilst not pregnant/lactating. The urine contained $12.4 \text{ g urea-N l}^{-1}$, $0.4 \text{ mg NO}_3^- \text{-N l}^{-1}$, $211 \text{ mg NH}_4^+ \text{-N l}^{-1}$, 5.5 mg P l^{-1} , 1.85 g Na l^{-1} , 13.9 g K l^{-1} and 284 mg Ca l^{-1} . The urine had a pH and EC of 8.48 and 25.3 mS cm^{-1} , respectively. Out of a total of 40 urine events between the 6 sheep, the mean and range of individual urine volumes were 104 (18–397) ml per urination event.

3.2. Weather data and soil moisture

The hourly rainfall, soil temperature (0–10 cm) and water-filled pore space (WFPS) data collected throughout the study period can be seen in Fig. 1. The DMPP application on the 29/06/15 for treatment SU + DMPP 4, was applied under warm and dry conditions. The DMPP application on the 13/07/15, for treatment SU + DMPP 2, was applied during a period of rainfall. The DMPP and urine applications (27/07/15) were also under periods of rainfall. The total rainfall over the course of the study was 137 mm, and the average soil temperature (0–10 cm) was 16°C , ranging from 11 to 20°C . The average WFPS over the course of the study was 27%, and ranged from 5 to 69%, with minimum values at the beginning of the experiment following an extended dry period, and maximum values occurring after rainfall events.

3.3. Nitrous oxide emissions

The fluxes of N_2O from all treatments can be seen in Fig. 2. When considering the whole automated chamber data set (8420 flux measurements), 79% of the headspace concentration data had an $R^2 > 0.95$, 88% had an $R^2 > 0.90$ and 97% of the data had an $R^2 > 0.80$, indicating the increase/decrease in N_2O within the chamber headspace over time fitted well to the assumption of linearity. In the vast majority of cases three, rather than four, data points of increase in chamber headspace concentration over time resulted in a higher R^2 . As can be seen in Fig. 2b, the main N_2O peak from the SU treatment occurred within the first nine days following urine application, with a maxima of $264 \pm 179 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. DMPP was effective in reducing this initial N_2O peak when applied at the same time as the urine (Fig. 2c), however, a broader, shallower peak began around the 30/08/15, which was not evident in the SU treatment. In both

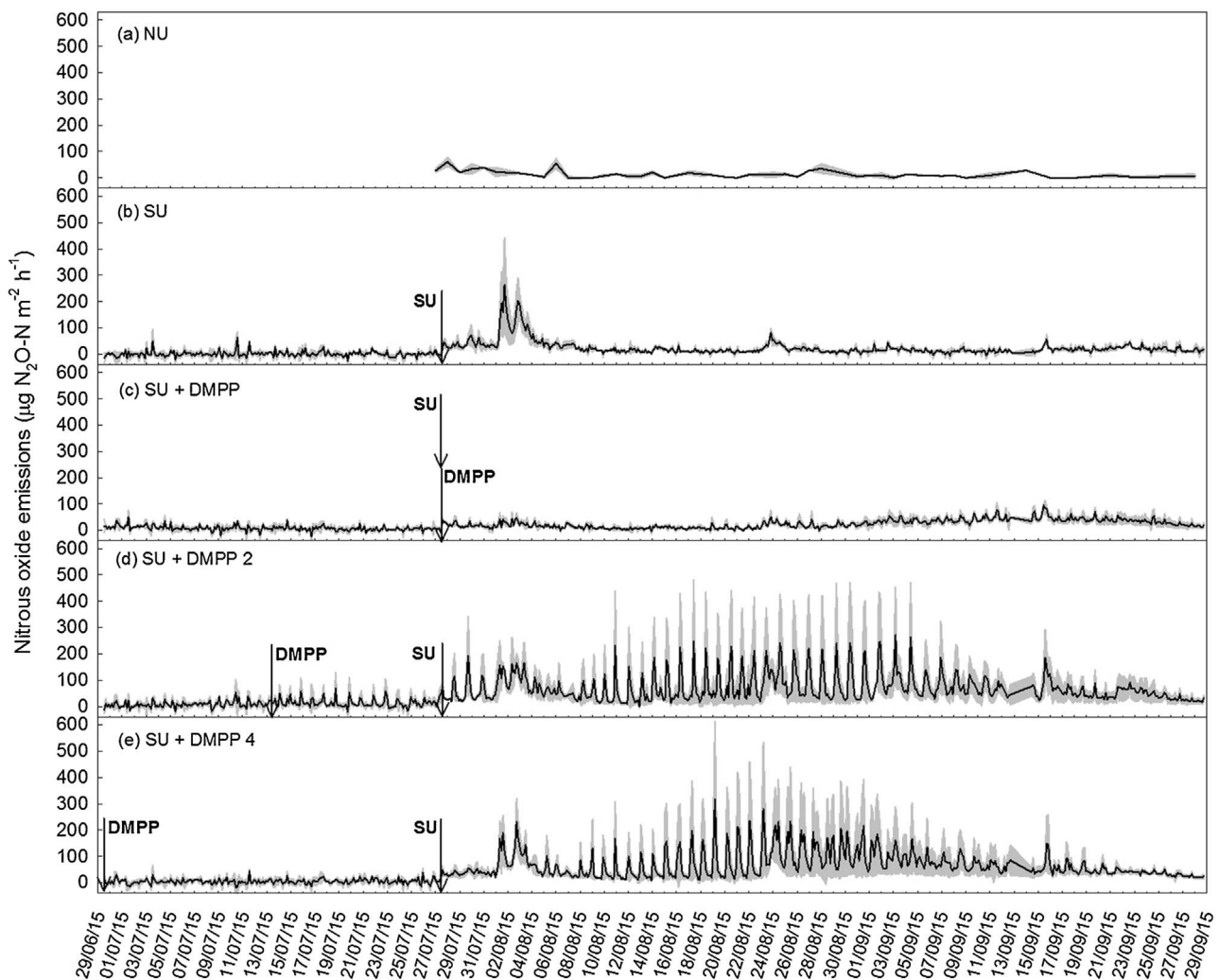


Fig. 2. N_2O fluxes following sheep urine and DMPP applications to a Eutric Cambisol, where panel a) displays fluxes from the NU treatment as measured by manual static chambers, panels b), c), d) and e) display fluxes from the automated chambers for treatments SU, SU + DMPP, SU + DMPP 2 and SU + DMPP 4, respectively. In each panel the black line represents the mean flux ($n = 3$) and the grey shaded area represents the upper and lower bounds of the SEM. Arrows indicate the timing of either sheep urine (SU) or DMPP applications and the dates along the x-axis are in dd/mm/yy format.

treatments, SU + DMPP 2 and SU + DMPP 4, this initial peak was similar in size (Fig. 2d and e, respectively) with maximum peaks in the first nine days of 192 ± 151 and $231 \pm 89 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$, respectively. Notably the variation in N_2O emissions is higher in Fig. 2d and e compared to Fig. 2b and c.

3.4. Cumulative N_2O emissions and emission factors

Cumulative N_2O emissions were calculated from the point of urine application onwards, and can be seen in Fig. 3. Mean cumulative emissions over 9 weeks ranged from 0.06 – $6.49 \text{ g N}_2\text{O-N m}^{-2}$ across all treatments. Particularly high emissions were observed within one replicate from both the SU + DMPP 2 and the SU + DMPP 4 treatments, where cumulative N_2O emissions were ca. 5 and 7 times larger than the mean of the remaining two replicates, respectively. Due to the observed extreme variability of cumulative N_2O emissions from SU + DMPP 2 and SU + DMPP 4, these treatments were not included in the ANOVA, due to large violations of the equality of variance assumptions; nevertheless, the results are shown in Fig. 3, as the data could shed light on spatial variability observed in soil N_2O emissions. In Fig. 3, it can be seen that the SU and SU + DMPP treatments emitted significantly more ($p < 0.01$) N_2O in comparison to NU. In contrast to our hypotheses, DMPP did not reduce the cumulative N_2O emissions from sheep urine patches when applied at the same time as the urine.

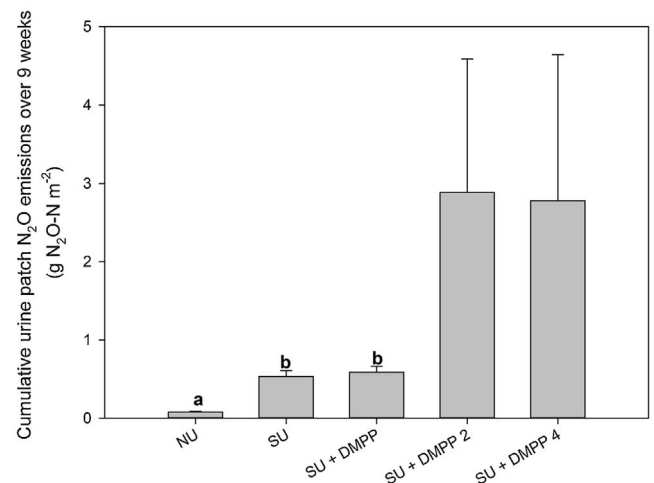


Fig. 3. Cumulative nitrous oxide emissions ($\text{g N}_2\text{O-N m}^{-2}$) over 9 weeks post urine and DMPP applications to a Eutric Cambisol. Bars represent arithmetic means ($n = 3$) and error bars denote SEM. Letters represent significant differences (one-way ANOVA with Tukey's post-hoc test; $p < 0.05$), where SU + DMPP 2 and SU + DMPP 4 were excluded from the analysis due to extremely high variability between replicates.

Although the SU + DMPP 2 and SU + DMPP 4 data show large variability and a relatively low number of replicates, based on the mean values, DMPP applied 2 and 4 weeks before urine application also appeared to be ineffective in reducing N_2O emissions from the urine patch (Fig. 3).

The urine patch N_2O emission factors over 9 weeks were 0.63 ± 0.10 , 0.70 ± 0.10 , 3.87 ± 2.36 and $3.73 \pm 2.57\%$ of the applied N for SU, SU + DMPP, SU + DMPP 2 and SU + DMPP 4 treatments, respectively. No significant differences ($p > 0.05$) were found between the amount of N lost as N_2O from the SU and SU + DMPP treatments. Emission factor values within the 'high-emitting' replicates within SU + DMPP 2 and SU + DMPP 4 treatments were as high as 8.20 and 8.84% of the N applied, respectively.

3.5. Diurnal nature of N_2O emissions

An analysis of the best three hour window (within a 24 h period) to sample fluxes from the chambers in this study was conducted, to determine the best time of day to sample chambers within a manual gas sampling campaign. As can be seen in Fig. S1 (Supplementary information), sampling between 09:00 in the morning and 12:00 noon most closely approximated the cumulative flux as measured by the automated system, where daily sampling within this time window would have underestimated the cumulative emissions by $0.22 \pm 3.51\%$ across all chambers ($n = 12$). Sampling between 18:00 and 21:00 was the next best time window, where emissions would have been overestimated by $1.60 \pm 3.83\%$. Sampling between 21:00 in the evening and 09:00 in the morning would have underestimated cumulative fluxes by a similar amount (cumulative fluxes underestimated by $7.49 \pm 3.63\%$, $7.10 \pm 4.66\%$, $8.09 \pm 4.71\%$ and $5.51 \pm 3.28\%$ at 00:00–03:00, 03:00–06:00, 06:00–09:00 and 21:00–24:00, respectively). Sampling between the hours of 12:00–18:00 would have overestimated cumulative fluxes ($22.46 \pm 5.05\%$ and $16.14 \pm 4.83\%$ at 12:00–15:00 and 15:00–18:00, respectively). These data show that N_2O fluxes across the whole experimental period were generally highest between the hours of 12.00 and 15.00 and lowest between 06:00 and 09:00.

In some chambers (e.g. 'high emitting' replicate from SU + DMPP 2 treatment), however, emissions were highest between ca. 00:30 and 06:30 (hh:mm), with minimum emissions in the diurnal cycle occurred between ca. 15:30 and 21:30 (hh:mm). An example of this is shown in Fig. S2 (Supplementary information) between 08/08/2015 and 14/08/15, where N_2O emissions appear to be out of phase with soil temperature. Within this individual chamber both N and labile C were present within the soil solution and assumed to not be limiting N_2O production. The maximum soil temperature over the period of observation shown in Fig. S2 was 26°C , and the minimum soil temperature was 12°C . The highest soil temperatures generally occurred at around 13:30 (hh:mm), however the minimum temperature occurred anywhere between 00:00 and 06:00 (hh:mm).

3.6. Mineral N dynamics

The extractable NH_4^+ and NO_3^- from soil cores in replicated plots can be seen in Fig. 4a and b, respectively, and the soil solution NH_4^+ and NO_3^- from Rhizon samples can be seen in Fig. 4c and d, respectively. In order to make the conversion between the mg kg^{-1} as expressed for the soil extract data, into mg l^{-1} of soil solution, a conversion factor of multiplying by ca. 3 is required (the exact conversion factor depends on the soil moisture at the time of sampling, therefore, conversion factors for each soil sampling date are supplied in Supplementary information: Table S1.0). Prior to urine application, no significant differences ($p > 0.05$) in extractable NH_4^+ concentrations were observed between treatments. On the day of urine application (27/07/15), all extractable NH_4^+ concentrations were greater than the control ($p < 0.01$), with no further differences between treatments. On

the second day following urine application, extractable NH_4^+ was only higher ($p < 0.05$) in the SU treatment in comparison to the control. Four days following urine application the extractable NH_4^+ was greater in all DMPP containing treatments ($p < 0.05$) compared to the control, but the SU treatment had returned to control levels ($p > 0.05$), indicating DMPP applications were delaying nitrification. One week following urine application the SU and SU + DMPP 4 treatments had returned to control levels ($p > 0.05$), however, the SU + DMPP and SU + DMPP 2 treatments had higher extractable NH_4^+ concentrations in comparison to the control ($p < 0.05$). This indicates that the effect of DMPP was shorter lived in the SU + DMPP 4, compared to SU + DMPP 2 and SU + DMPP treatments. No differences were observed in extractable NH_4^+ between treatments 10 days after urine application. Two weeks following urine application SU + DMPP had a higher extractable NH_4^+ content compared to the control ($p < 0.05$), and after 3 weeks extractable NH_4^+ was greater in SU + DMPP and the SU + DMPP 2 compared to the control ($p < 0.05$). No further differences in extractable NH_4^+ concentrations were observed beyond three weeks following urine application ($p > 0.05$).

No differences were observed in extractable NO_3^- concentrations between treatments prior to, on the day of urine application, or two and four days following urine application ($p > 0.05$). After one week, however, all urine containing treatments had a higher extractable NO_3^- content in comparison to NU ($p < 0.001$). Ten days following urine application the extractable NO_3^- concentrations were still greater than NU in all urine containing treatments. Here, the SU had a higher extractable NO_3^- concentration than the SU + DMPP treatment, but not the SU + DMPP 2 or SU + DMPP 4 treatments, indicating a delay in nitrification when DMPP was applied at the same time as the urine only. Two weeks after urine application, extractable NO_3^- was greater in all urine containing treatments compared to NU, but no other differences were detected beyond this date ($p > 0.05$).

Rhizon soil solution samples were only obtained when soil conditions were moist enough, therefore, n varied on each sample date, depending on whether sufficient sample was obtained for analysis. Soil solution NH_4^+ concentrations in the treatments containing sheep urine appeared to return to control values at a similar time to the soil extracts. The soil solution NO_3^- concentrations increased steadily during the 9 d following urine application. At the point of 9 d following urine application, the soil solution NO_3^- followed the numerical trend $\text{NU} < \text{SU} + \text{DMPP} < \text{SU} + \text{DMPP 2} < \text{SU} + \text{DMPP 4} < \text{SU}$, which indicates DMPP delayed nitrification rates compared to sheep urine alone, but was less effective the longer the time since DMPP application. Notably, the soil solution NO_3^- concentrations were numerically higher than NU for a longer period in comparison to the extractable NO_3^- data, indicating a difference in N processing rates between duplicated plots and the chambers.

3.7. Dissolved organic C and N dynamics

The total extractable N and dissolved organic C during the course of the field trial can be seen in Fig. 5a and b, respectively; the total N and dissolved organic C measured in the soil solution samples can be seen in Fig. 5c and d, respectively. Differences between total extractable N were only found at one sampling point following urine application to the plots, this was at two weeks following urine application where the SU + DMPP extractable N was greater than the control ($p < 0.05$). Similarly, not many differences in extractable dissolved organic C were found between the treatments at the different sampling points. Here, the only differences found were two days following urine application, where SU and SU + DMPP 2 had higher levels of extractable dissolved organic C compared to the control ($p < 0.05$).

The soil solution N increased following urine application, and remained numerically higher than the control until the end of the study, which reflected the remaining NO_3^- in the soil solution of the chamber plots. In the two chambers which emitted the highest amounts

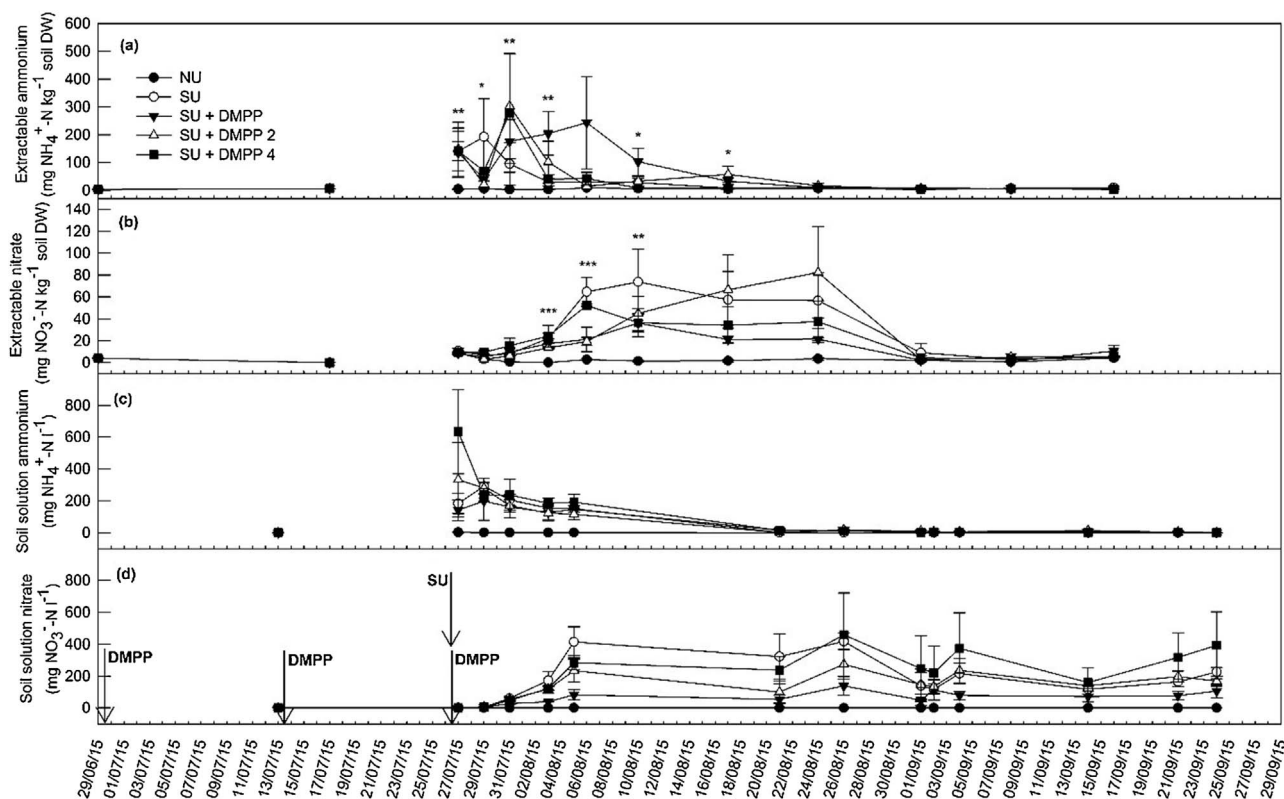


Fig. 4. Mineral nitrogen dynamics during the course of the field trial. Extractable ammonium-N can be seen in panel a) and extractable nitrate-N in panel b), which were measured on extracted soils from replicated plots (outside chambers). The soil solution ammonium-N is shown in panel c) and soil solution nitrate-N in panel d), which were measured from Rhizon samples located within the chambers. The figure legend applies to all panels, symbols represent means ($n = 3$), and error bars denote SEM. Arrows in panel d) represent timings of sheep urine (SU) and/or DMPP applications to the respective treatments. Asterisks above symbols represent level of significant differences (ANOVA) and was conducted for data in panels a) and b) only. The date format along the x-axis is dd/mm/yy.

of N_2O , it was found that the soil solution dissolved organic C concentration was ca. 2 and 4 times higher than the other samples collected across the plots, prior to urine application. The soil solution dissolved organic C concentration increased following urine application and treatments appeared to decline at similar rates. No observable differences in soil solution dissolved organic C were found between treatments after ca. 3 weeks following urine deposition.

3.8. Pasture biomass and foliar N content

The foliar N content measured at block level ($n = 3$), was $3.5 \pm 0.5\%$ prior to urine application. No significant differences ($p > 0.05$) in foliar N content were observed between treatments 3 weeks following urine application, where the mean foliar N content across all treatments was $3.4 \pm 0.2\%$. The urine-containing treatments all had a significantly greater pasture foliar N content in comparison to the control ($p < 0.01$) at 6 and 9 weeks following urine application, where the mean foliar N content of all the urine-containing treatments was $4.3 \pm 0.1\%$ and $4.6 \pm 0.2\%$, compared to the control value of $3.2 \pm 0.2\%$ and $3.0 \pm 0.4\%$, respectively. No significant differences ($p > 0.05$) were found for pasture biomass in the plots at 3, 6 or 9 weeks following urine application, where the mean pasture biomass across all samples were 0.47 ± 0.2 , 0.51 ± 0.03 and 0.29 ± 0.04 t DM ha^{-1} , respectively.

4. Discussion

4.1. Effect of DMPP on sheep urine patch N_2O emissions and mineral N dynamics

In this study, a liquid DMPP application (1 kg ha^{-1}) did not reduce

cumulative N_2O emissions from sheep urine patches in a temperate grassland under summer conditions, when applied at the same time as the urine. Whilst no significant effect of DMPP was found for cumulative N_2O emissions, there was an observable effect of the nitrification inhibitor based on temporal trends in the N_2O fluxes and mineral N data. When considering the SU + DMPP treatment, the initial N_2O peak following urine application was much reduced in comparison to the SU-only treatment, however, a broader, shallower peak towards the end of the measurement period was observed. In addition, the extractable NH_4^+ concentrations were higher in the SU + DMPP and SU + DMPP 2 treatments up to three weeks following urine application. This suggests that nitrification rates were delayed, with the effect being greater the shorter the time since DMPP application. Whilst some effect of DMPP was observable, this tended to delay, rather than reduce overall N_2O emissions in this study. The N application in the sheep urine patches was relatively high at 725 kg N ha^{-1} , yet Di and Cameron (2012) found DMPP reduced N_2O emissions by 66% from cattle urine N applications as high as $1000 \text{ kg N ha}^{-1}$, although a higher DMPP application of 5 kg ha^{-1} was used in this study.

There could be several reasons for not observing an effect of DMPP on cumulative N_2O emissions. It could be due to the DMPP load applied (1 kg ha^{-1}), which was low compared to other studies e.g. 5 kg ha^{-1} application in Di and Cameron (2012) and 10 kg ha^{-1} application in the laboratory study of Di and Cameron (2011). It could also be due to high soil temperatures, which may have resulted in rapid DMPP degradation by microbial activity. Menéndez et al. (2012) and Vasquez et al. (unpubl.), however, found no significant effect of increasing temperature between 10 and 20°C on DMPP degradation, which represents the typical range of soil temperatures observed in this field trial. The soil water content has also been identified as an important

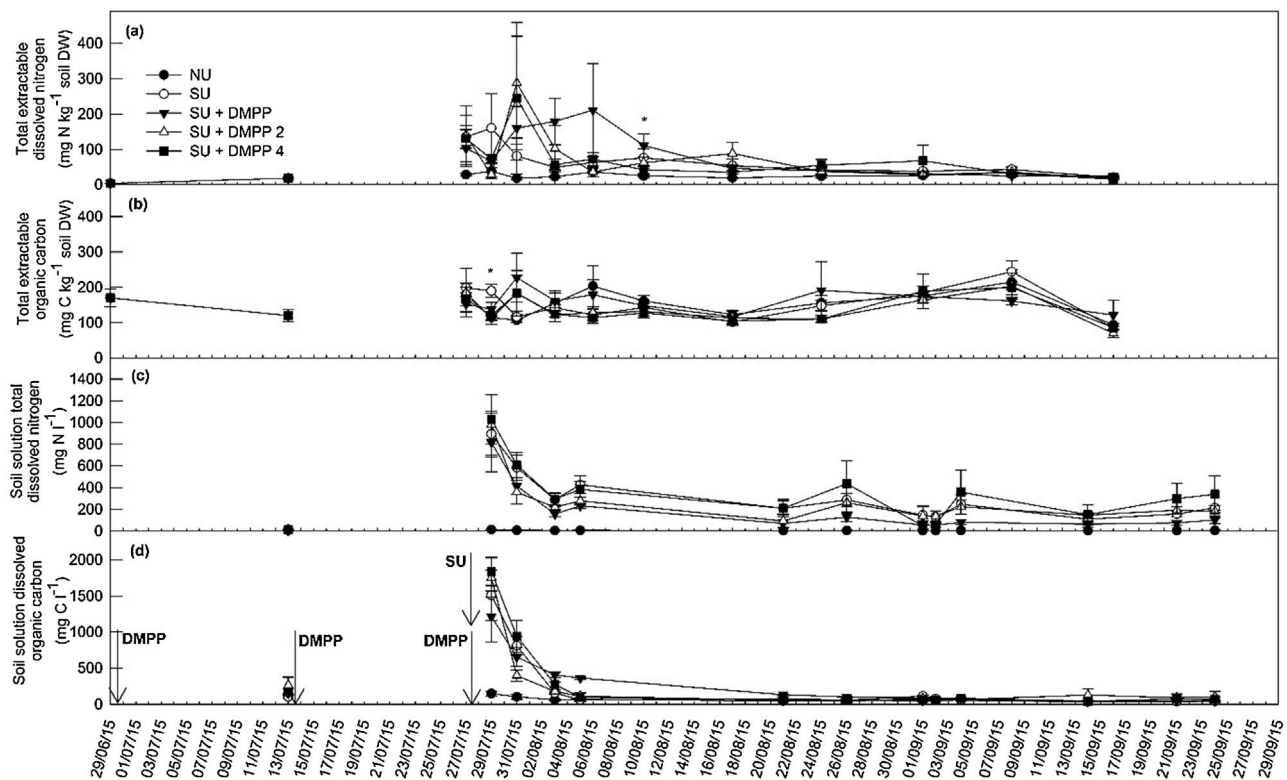


Fig. 5. Total extractable and soil solution dissolved N and C dynamics. Total extractable dissolved N is displayed in panel a) and total extractable dissolved organic C is displayed in panel b), which were measured on extracted soils from replicated plots (outside chambers). Soil solution dissolved N is displayed in panel c) and soil solution dissolved organic C is displayed in panel d), which were measured using Rhizon samplers located within the chambers. The figure legend applies to all panels, symbols represent means ($n = 3$), and error bars denote SEM. Arrows in panel d) represent timings of sheep urine (SU) and/or DMPP applications to the respective treatments. Asterisks above symbols represent level of significant differences (ANOVA) and was conducted for data in panels a) and b) only. The date along the x-axis is expressed in dd/mm/yy.

parameter influencing the persistence of this molecule in soil, where a lower water content (and hence greater oxygen content) may increase oxidation of the molecule (Chen et al., 2010; Menéndez et al., 2012). In this study, the maximum observed water-filled pore space was 69%, but more often it remained between 20 and 40%, which suggests depletion of DMPP via oxidation may have occurred. Further depletion of DMPP from the soil solution could have occurred due to immobilisation within non-target microbial biomass, sorption to the soil's solid phase and root surfaces or canopy interception of the applied inhibitor.

Another possible reason for the lack of effect of DMPP on cumulative N_2O emissions could be inefficient mixing with the soil solution and urine-N. Given that the DMPP was applied under rainfall conditions in the treatments SU + DMPP and SU + DMPP 2, and that there was an observable effect of DMPP on nitrification rates and the temporal nature of N_2O fluxes in SU + DMPP treatment, it would suggest that the inhibitor at least partially mixed with the urine derived N. The DMPP applied 4 weeks before urine application, however, may have experienced greater degradation or pasture canopy interception during application, as it was not applied during a rainfall event. It cannot be excluded that weather conditions during the time of DMPP applications may have confounded observations of a treatment effect. Improvements to the experimental design could have been made via the use of rain shelters and/or irrigation systems to maintain standard conditions throughout the study period. Monitoring N_2O emissions for 9 weeks following urine application may have also not been a long enough measurement period for treatment effects to be observed.

Further research based on different DMPP application methods is required, e.g. investigating whether DMPP would have been more effective if mixed with the urine prior to application to soil. Comparatively more research has been conducted on different application methods of DCD in comparison to DMPP (e.g. Ledgard et al., 2008; Minet et al., 2013; Wakelin et al., 2013; Luo et al., 2015). This may be

due to the original commercial development of DMPP as a fertiliser coating (Pasda et al., 2001; Zerulla et al., 2001), rather than for its use on urine-influenced soils.

4.2. N_2O emission factors

The 9-week emission factor for the SU treatment was $0.63 \pm 0.01\%$ of the applied N, which is lower than the default IPCC emission factor for sheep excreta of 1% (IPCC, 2007). This is consistent with reported emission factors being lower under spring-summer conditions in comparison to autumn-winter (Allen et al., 1996). No significant differences were found for N_2O emission factors between SU and SU + DMPP. The mean 9 week N_2O emission factor across all urine receiving treatments (two extreme values removed as values were > 1.5 times the interquartile range; $n = 10$) was $0.98 \pm 0.30\%$ of the applied N. This is remarkably similar to the default N_2O emission factor proposed by the IPCC of 1%. The highest emission factors occurred in the SU + DMPP 2 and SU + DMPP 4 treatments (8.20 and 8.84%, respectively). Whilst these are remarkably high values, results of a similar magnitude have been reported in De Klein and van Logtestijn (1994). It is suggested that the overlapping of nutrient hotspots greatly increased the emission factors for sheep urine. As the study was fenced off from grazing livestock, we suggest the nutrient hotspot may have come from a source other than a urine patch (see Supplementary information S1.0, for additional discussion).

4.3. Spatial and temporal variability in N_2O fluxes

N_2O emissions from agricultural soils are notoriously variable, both in time and space (Hénault et al., 2012), and this study proved to be no exception. Notably the SU and the SU + DMPP treatments did not have as high a variability in N_2O emissions as the SU + DMPP 2 and SU

+ DMPP 4 treatments (Figs. 2 and 3). Due to this we can conclude with more confidence that DMPP had no effect on cumulative N_2O emissions when applied at the same time as the urine, under these experimental conditions. Bearing this in mind, it is unlikely that the DMPP would have had an appreciable effect on reducing N_2O emissions when DMPP was applied weeks before the urine, even if the data had not suffered from the large spatial variability.

Whilst a high temporal variability was observed, the automated chamber system captured this variability, thereby reducing bias in emission calculations due to sampling time and frequency, which can be an artefact of manually sampled static chamber based campaigns (e.g. Yamulki et al., 2001; Scheer et al., 2014). The N_2O emissions in this study were also, however, highly spatially variable. Here, one replicate in each of the treatments SU + DMPP 2 and SU + DMPP 4 demonstrated a marked difference to their respective replicates. Future studies should consider maximising the number of replicates in order to capture spatial variability in N_2O emissions, when using an automated chamber based system. This will potentially result in a having to select fewer treatments for investigation, however, would be appropriate where emission factors are to be measured for inventory purposes.

4.4. Diurnal nature of N_2O fluxes

The existence of diurnal trends in N_2O flux data have been identified in several studies (e.g. Thomson et al., 1997; Williams et al., 1999; Yamulki et al., 2000, 2001; Hyde et al., 2005a, 2005b; Hatch et al., 2005; Alves et al., 2012; Scheer et al., 2014; Shurpali et al., 2016; Wei et al., 2016), yet contrasting timings of daily minimum and maximum N_2O flux are reported between studies. When considering the current study, the best time of day to manually sample chambers in order to best represent emissions from the automated system, would have been between 09:00 and 12:00. This is in agreement with Rochette et al. (2015) and similar to the recommended sampling time of 09:00–10:00 as found in Alves et al. (2012). Scheer et al. (2014) found the diurnal nature of N_2O fluxes to be greatest when the mineral N and moisture were non-limiting. This appeared to be true in our study, and the diurnal trend was also strongest in the chambers where urine was applied to a dissolved organic C hotspot.

Large overestimations of the cumulative flux would have occurred if sampling was conducted between 12:00–15:00 and 15:00–18:00, suggesting maximum diurnal emissions generally occurred within these time frames over the study period. This was not the case for all chambers at all times though, and in some chambers maximum diurnal N_2O fluxes occurred at night time and early morning. The existence of these contrasting diurnal trends, even within the same study, have also been shown by Shurpali et al. (2016), where N_2O emissions had maximum peaks during both the day and the night at different times. In Shurpali et al. (2016) night-time diurnal peaks occurred when N was limiting and the magnitude of N_2O flux was low, however, in the current study emissions were greater at night when N and C were presumably non-limiting, following the urine application.

Some studies show N_2O emissions to be closely in phase with soil temperature (e.g. Williams et al., 1999; Hatch et al., 2005), whilst others observe a greater lag time between soil temperature and N_2O production. The observed variation in timings of minimum and maximum daily flux could be due to the fact that N_2O emissions are dependent on several factors (not just soil temperature), which may interact in different ways to produce different timings of minimum and maximum flux. The solubility and microbial consumption of O_2 increases alongside temperature, which may lead to reductions in N_2O due to the occurrence of complete denitrification (Yamulki et al., 2000). The pattern of N_2O production and consumption across soil depth may also vary with temperature, where maximum and minimum temperatures would occur later at soil depth. Another possible reason is that greater compaction could result in slower gas diffusivity (Ball et al., 1999; Sitaula et al., 2000) from the source of N_2O to the chamber

headspace i.e. a shorter lag between maximum temperature and maximum N_2O flux was found in the sieved and repacked soils of Hatch et al. (2005), as well as soils which had been subjected to rotary hoeing during incorporation of vegetable residues in Scheer et al. (2014). This area of study requires further research as it has implications for recommendations of the best time to sample N_2O emissions over a 24 h period to reflect the average daily fluxes, and to more accurately calculate N_2O losses from soils (Shurpali et al., 2016). It also supports the use of methods with a high temporal resolution for monitoring N_2O emissions, where possible.

4.5. Destructive vs. non-destructive soil sampling for measuring soil mineral N pools

In this study, differences were found between the mineral N dynamics as measured by taking soil cores from duplicate urine patches (destructive) and as measured in the soil solution of the chambers (non-destructive). The soil solution NO_3^- concentrations in the urine treated chambers remained higher than the control for a longer period than the extractable NO_3^- , therefore, further N_2O emissions could have occurred beyond the duration of this study. The differences in N processing found between the duplicated urine patches may reflect artefacts of coring the duplicated urine patches. This may have influenced the aeration and water infiltration dynamics in these areas. Conversely, the chambers may have caused more shading and/or interception of rainfall in comparison to the duplicated plots. The difference in N processing rates highlights the need for the development and use of non-destructive sampling techniques for use inside chambers. Rhizon samplers were useful in this study for determining differences, which would not have been observed from only taking soil cores from duplicated urine patches. Limitations of the Rhizons were that successful sample collection depended on sufficient soil moisture, which sometimes resulted in a variable number of replicates on different sample dates. Deploying multiple Rhizons within a urine patch could potentially overcome the issue of the occasional small sample volume. Nevertheless, Rhizon samplers would be ineffective under extended dry periods.

5. Conclusions

A recent review by Gilsanz et al. (2016), highlights the paucity of studies regarding DMPP efficacy in reducing N_2O emissions, in comparison to the well-researched inhibitor DCD. This is especially true of urine-influenced soils, where this study contributes new information for DMPP efficacy in sheep urine patches, under temperate summer conditions. In this study, DMPP applied at a rate of 1 kg ha^{-1} influenced the dynamics of N processing rates, but had no effect on overall cumulative N_2O emissions or emission factors. Care should be taken when interpreting these results, due to a low number of treatment replicates within this study and the short N_2O measurement period. In order to be able to assess the practicality and cost-effectiveness of DMPP use in temperate grazed grasslands, further information is required on the loading rates of DMPP required to reduce N_2O emissions from urine patches under summer conditions and whether different application methods alter the efficacy of this nitrification inhibitor. Whilst the duration of DMPP efficacy remained unestablished in this study, this is an important factor to determine, due to the potential of applying inhibitors to urine patches of varying ages.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2017.05.017>.

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